Motor-cargo adaptors at the organelle-cytoskeleton interface

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Abstract

Cytoskeletal motors of the dynein, kinesin and myosin superfamilies maintain and adapt subcellular organelle organization to meet functional demands and support the vesicular transport of material between organelles. These motors require the capacity to specifically recognize the vesicle/organelle to be transported and are capable of selective recognition of multiple cargo. Recent studies have begun to uncover the molecular basis for motor recruitment and have highlighted the role of organelle-associated 'cargo-adaptor' proteins in cellular transport. These adaptors possess sequences and/or structural features that enable both motor recruitment and activation from regulated, inactive, states to enable motility on the cytoskeleton. Motor-cargo adaptor interactions define a key organelle-cytoskeleton interface, acting as crucial regulatory hubs to enable the cell to finely control membrane trafficking and organelle dynamics. Understanding the molecular basis of these interactions may offer new opportunities to control and manipulate cytoskeletal and organelle dynamics for the development of new research tools and potentially therapeutics.

Introduction

Our understanding of the molecular basis for cargo attachment/recognition by cytoskeletal motor proteins has historically lagged behind that of the motility mechanisms that enable ATP hydrolysis and translocation on the actin or microtubule cytoskeletons. Cells support a vast range of organelle and vesicular transport processes with a relatively limited complement of motors, highlighting the requirement for the selective recognition of multiple cargo [1]. This is perhaps most apparent in the case of cytoplasmic dynein-1 (dynein hereafter) which carries out the bulk of microtubule minus-end directed organelle transport in mammalian cells [2]. Even in the case of the larger and more diverse kinesin and myosin superfamilies, it is clear that one motor will transport more than one cargo and that certain family members play more important roles in organelle transport than others [3-5].

Until relatively recently we lacked detailed understanding of the general principles that enable recognition of multiple cargo. New insights at the structural level into motor-cargo interfaces have begun to reveal how a group of proteins, known as 'cargo-adaptors', enable selective motor recruitment [6,7]. Loosely defined as molecules that have the capacity to interact directly or indirectly with specific organelle(s) and directly with a motor protein complex, these adaptors act as molecular bridges between cargo and the cytoskeleton. Recruitment of adaptors themselves to organelles can occur through a range of mechanisms; adaptors may be transmembrane proteins like Nesprin-2 or Calsyntenin-1[8-10], interact directly with membrane lipids, or very often, are recruited by small GTPases, placing them under the control of nucleotide exchange factors and GTPase activating proteins. A recently described pathway for kinesin-1 recruitment to lysosomes illustrates this. The BORC complex, recruits the small GTPase Arl8, which interacts with the cargo adaptors SKIP(PLEKHM2). SKIP interacts directly with kinesin-1[11,12]. BORC itself interacts with the lysosome associated nutrient signaling machinery to control lysosome transport in response to amino acid availability[13]. Similarly, the dynein adaptor BICD2 can be recruited to vesicles by Rab6 [14].

Cargo adaptor - motor binding interactions can help to drive motors from inactive regulated states to active states capable of motility on the microtubule or actin cytoskeleton making them critical regulatory hubs for organelle transport. Moreover, one adaptor may also work in concert with others, and/or engage a motor at multiple sites to recruit and promote activity, and potentially coordinate with direct recognition of lipid components of the organelle membrane. In this review, we will consider recent progress in our understanding of how these adaptors engage motor proteins for three of the better characterized processive organelle transporting motors; kinesin-1, myosin-V and cytoplasmic dynein-1. We will focus on where recent structural studies have begun to reveal the molecular details of the organelle-cytoskeleton interface and suggest that some common themes for motor recruitment are now beginning to be established that may hold the key to understanding how the vast complexity of intracellular transport is orchestrated.

The recognition of activating adaptor peptide motifs by kinesin-1 and myosin-V

Proteins or protein complexes involved in 'cargo selection' frequently recognize short peptide motifs. Examples include binding of acidic-dileucine motifs by clathrin adaptor complexes to select membrane cargo for endocytosis or recognition of mono- or bipartite basic motifs by importins for nuclear import. Peptide motifs often occur in relatively unstructured regions of proteins and bind to specific surfaces on the receptor, allowing one piece of machinery to recognize multiple cargoes. From an evolutionary perspective this recognition mechanism has the potential to allow cargo to easily gain or lose the capacity to interact with transport machinery. Given this, it is perhaps surprising that such principles have taken time to emerge for motor proteins. Recently, however, it has become clear that the processive actin motor myosin-V operates such a mechanism to help enable cargo recognition in at least 2 distinct contexts **(Figure 1A)**. The globular tail domain (GTD) of myosin-V binds related sequences in the Melanophilin (MLPH), Spir-1, and Spir-2 adaptors which are important for the transport of melanosomes and Rab11 positive endosomes

respectively [15-19] **(Figure 1a-c).** In the case of MLPH, attachment to the membrane is via Rab27a, and in the case of Spir-2, via interactions with Rab11 and direct membrane binding by its FYVE domain. Both proteins contain sequences of approximately 25 amino acids, known as globular tail binding motifs (GTBM), that comprise basic and acidic flanks with a predominantly hydrophobic core and bind to the same site on subdomain 1 of the GTD of myosin-Va with low micromolar/high nanomolar affinity, albeit with slightly different modes of interaction **(Figure 1b,c)**. Although as yet not structurally defined, the secretory granule adaptor Granuphilin likely binds to the same site using a related mechanism [16]**.** It appears that this motif recognition mechanism can provide isoform selectivity between the 3 myosin-V isoforms present in vertebrates (myosin-Va, myosin-Vb and myosin-Vc); whilst Spir-1/2 GTBM binds to both myosin-Va and Vb, MLPH GTD binds only to myosin-Va with high affinity [16]. This motif recognition mechanism does not operate in isolation but instead cooperates with other protein-protein interactions to promote selective motor recruitment. The myosin-Va GTD also interacts directly with Rab11 on the vesicle membrane and the binding of Spir-2 promotes this association in cells [15]**.** Similarly, MLPH interacts with the exon F region of myosin-Va that precedes the GTD [17]**.** When not engaged in transport motors are frequently found in inhibited conformations and require cargo adaptor binding interactions for activation. Recognition of the MLPH is sufficient to activate myosin-Va in vitro [20] by inhibiting regulatory interactions between the GTD and head domains, potentially via an allosteric mechanism that inhibits GTD dimer contacts maintained in the inhibited state [15,21] **(Figure 1a).**

The notion of recognition of an activating peptide motif by a globular tail domain can be extended to kinesin-1 **(Figure 1,d-f).** The heterotetrameric form of the motor consists of two heavy chains (KHCs) and 2 light chains (KLCs) and, like myosin-V, exists in a folded, regulated conformation in the absence of cargo binding [3]. The light chains of kinesin-1 contain important cargo binding sites on their tetratricopeptide repeat (TPR) domains which can recognize at least 2 distinct classes of adaptor peptide motifs with low micromolar affinity **(Figure 1, e,f)** . The first, known as W-acidic, has the consensus L/M-D/E-W-D/E and can interact with both the KLC1 and KLC2 isoforms while the second, Y-acidic,

consensus D-ϕ-Y-ϕ-E (where ϕ indicates a hydrophobic residue), is selective for KLC1. These adaptors bind at overlapping sites on the highly basic concave surface of the TPR resulting in an induced fit adaption in its curvature. Key binding contacts for both motifs involve sequence specific interactions between L/M, W and Y residues which occupy relatively hydrophobic pockets on the TPR surface and a network of salt bridges and hydrogen bonds [22,23]**.** The plasticity of the TPR domain and the intrinsic flexibility of these cargo adaptor peptides makes it unlikely that these two motifs define the full extent of recognition by the TPR groove, hinting that other modes of peptide binding wait to be discovered.

Notably, binding of the W-acidic motif appears sufficient to promote kinesin-1 activity in a cellular context, with fusion of these sequences to integral membrane proteins promoting transport of the associated compartment [11,24-26]. In contrast, binding of the Y-acidic class does not [24], and instead requires the support of other interacting partners [27]**.** We have provided data that suggests this is likely due to the capacity of the W-acidic but not the Y-acidic motif to displace an autoinhibitory intramolecular interaction between the KLC TPR domain and the unstructured region immediately N-terminal to it [28,29]**,** with downstream effects on inhibitory interactions within the KHCs [29].

Thus, it appears that despite an absence of sequence or structural relationships, the engagement of loose consensus peptide motifs, that are shared between multiple cargo adaptors, play remarkably similar roles for both cargo recognition and activation of myosin-V and kinesin-1.

A common role for coiled-coil adaptors and the recruitment/co-ordination of multiple motors?

Recent studies have also highlighted the binding of dimeric coiled coils as a second mode of adaptor recognition utilized by myosin-V, kinesin-1 and dynein. The kinesin-1 light chain TPR domain can interact directly with a leucine zipper domain (a subclass of coiled-coil) within the JIP3 adaptor protein. This cooperates with peptide motif recognition by JIP1 [30-32] **(Figure 2a).** Both proteins also directly engage the KHCs [33,34]**.** JIP3 may promote dimerization

of the kinesin light chain TPR domains within the tetramer to promote activation [30]. Alternatively, this could provide a mechanism to cross-link multiple motors into a larger assembly, analogous to the recruitment of multiple dyneins discussed below **(Figure 2b)**. Another adaptor which interacts with KLC via a coiled coil region is FYCOI, a protein involved in transport of autophagic vesicles, although the structural basis is yet to be established [35,36]. Similarly, the GTD of myosin-V interacts with a 4 helix bundle of RILPL2 that is stabilized by a dimeric coiled-coil, bringing together two cargo recognition domains. RILPL2 was co-crystalized with the GTBM of MLPH [16] and, while it is not clear whether this specific ternary complex (RILPL2-GTD-MLPH) exists in cells, this nonetheless illustrates how binding of coiled-coil containing adaptors could cooperate with peptide motif recognition to facilitate motor recruitment, and further underscores similarity in the cargo recruitment mechanisms of myosin-V and kinesin-1.

The most striking example of where this structural feature is employed is in the recruitment and activation of dynein/dynactin. This has been extensively reviewed recently [2] so we will limit the discussion here to just some key points. A series of dimeric proteins have been identified as activating adaptors, which have the capacity to bind cargo and possess an extended coiled coil that binds along the length of the dynactin filament and interacts with the dynein heavy chains (DHC) [37-40] **(Figure 2d).** These adaptors also interact with 'Helix-1' of dynein light intermediate chains (LICs) using Hook domains, EF hand domains or further coiled-coil sequences to enhance processivity [41,42]. Moreover, certain adaptors can promote the recruitment of more than one dynein complex to increase force, speed and processivity [40,43]. It is interesting to note that coiled-coil containing activating dynein adaptors/interacting proteins including HOOK3, HAP1, TRAK1, BICD2, JIP3 and RILP also have the capacity to interact with several kinesins [44-47]. In the case of the latter two, JIP3 and RILP, coiled-coil sequences are relatively short and so their mode of interaction likely differs from previous four, an element of which includes interaction with the p150glued component of the dynactin complex [48,49]. RILP (late endosomes/lysosomes) is closely related to RILPL2 (RILP-like 2) which can recruit myosin-V. It therefore seems possible that dimeric coiled-

coil proteins provide a useful platform to engage dimeric motors, not just for the recruitment and activation of dynein-dynactin, but also may allow the grafting of features that enable the coordinated recruitment of opposite polarity motors for bi-directional transport on the microtubule network, and possibly the coordination of transport between the microtubule and actin cytoskeletons.

Synergistic recognition of membrane lipids and protein adaptors

In addition to protein recognition motifs, an important component of organelle identity is the lipid composition of the membrane. It is clear that at least some motors have the capacity to directly recognize both protein and lipid determinants, potentially as part of a co-incidence detection mechanism. For example, the cargo binding domain of myosin-X is composed of 3 tandem PH domains that can bind to the phosphoinositides $PI(3,5)P_2$ and $PI(3,4,5)_2$ [50] and a MyTH4-FERM domain that can specifically bind an alpha helical motif from the cytoplasmic tail of the membrane receptor DCC (deleted in colorectal cancer)[51] [52]. Similarly, the PH and PX domains of kinesin-3 family members can specifically bind to phospholipids [53-56]. The heavy chain subunit of kinesin-1 (Kif5B) was recently shown to interact with $PI(4,5)P_2$ to promote autolysosome tubulation [57]. The dynein-dynactin complex has been proposed to interact with membranes via spectrin proteins which bind acidic phospholipids via a PH domain and can directly bind the Arp filament in dynactin [58] and ankyrin B which can interact with PI(3)P and the p62 subunit of dynactin[59]. Going forward, it will be important to understand these indirect membrane interactions in the context of recent structural studies on the dynein-dynactin complex and whether direct engagement of the organelle membrane by motor protein complexes is a more general feature of cargo recruitment and recognition that cooperates with the binding of organelle-associated (activating) protein adaptors.

New opportunities to control transport

The studies described above have begun to understand these important organelle-cytoskeleton interfaces in fine molecular detail. Since these are

frequently not simply sites of attachment but also allosteric regulators of motor activity, an understanding of adaptor-motor interactions may offer new opportunities and targets to manipulate motor function, and consequently, cytoskeletal and organelle dynamics. Because cargo recognition mechanisms are quite divergent within motor protein families, this may help to avoid issues of specificity when targeting ATPase activities in more highly conserved motor domains. These would be valuable as research tools and possibility as therapeutics, particularly where transport is dysregulated in neurological disease or hijacked by pathogens. We have recently described a small-molecule that targets the kinesin-1 peptide motif recognition interaction (discussed above) to promote its activity. The major consequence appears to be activation of the secondary function of this motor in controlling microtubule organization through microtubule-microtubule sliding [60,61]. **Figure 3** [60] shows the effect of this compound on microtubule organization in HAP1 cells, promoting microtubule bundling, looping and the formation of microtubule based cellular processes. Going forward, it will be important to understand how this idea can be applied to other motors and crucially whether it might be possible to target transport of specific (or specific classes of) cargo over others to achieve fine control of transport, or whether it is possible to enhance motor activity in disease states when transport is impaired.

Concluding remarks

It is becoming clear that understanding motor-cargo recognition mechanisms lies at the heart of understanding the dynamic organization of the cell and may provide us with new opportunities to control and manipulate it. As we dissect the molecular basis for motor-cargo interactions we are beginning to learn about the 'rules' that govern the recruitment and activation of specific motors, and so enable a single motor to function in different contexts. Moreover, despite differences between mechanisms, some general trends have begun to emerge. This is perhaps not surprising given that efficient transport relies on coordination of the activity of multiple motors. Progress in understanding mechanisms of motor recruitment prompts the question of how transport is terminated. This may be controlled at the level of GTPase mediated recruitment

of adaptors themselves or through additional factors dissociate adaptor and motor[62]. The challenge ahead is to integrate these snapshots into a better understanding of how multiple motors and their regualtors work together to control organelle dynamics and cellular organization.

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References

1. Vale RD: **The molecular motor toolbox for intracellular transport**. *Cell* 2003, **112**:467–480.

2. Reck-Peterson SL, Redwine WB, Vale RD, Carter AP: **The cytoplasmic dynein transport machinery and its many cargoes.** *Nature Reviews Molecular Cell Biology* 2018, **19**:382–398.

3. Verhey KJ, Hammond JW: **Traffic control: regulation of kinesin motors**. *Nature Reviews Molecular Cell Biology* 2009, **10**:765–777.

4. Hirokawa N, Noda Y, Tanaka Y, Niwa S: **Cytoskeletal motors: Kinesin superfamily motor proteins and intracellular transport**. *Nature Reviews Molecular Cell Biology* 2009, **10**:682–696.

5. Hartman MA, Spudich JA: **The myosin superfamily at a glance.** *Journal of Cell Science* 2012, **125**:1627–1632.

6. Fu M-M, Holzbaur ELF: **Integrated regulation of motor-driven organelle transport by scaffolding proteins.** *Trends Cell Biol.* 2014, **24**:564–574.

7. Akhmanova A, Hammer JA: **Linking molecular motors to membrane cargo**. *Curr. Opin. Cell Biol.* 2010, **22**:479–487.

8. Wilson MH, Holzbaur ELF: **Nesprins anchor kinesin-1 motors to the nucleus to drive nuclear distribution in muscle cells.** *Development* 2015, **142**:218–228.

9. Konecna A, Frischknecht R, Kinter J, Ludwig A, Steuble M, Meskenaite V, Indermühle M, Engel M, Cen C, Mateos J-M, et al.: **Calsyntenin-1 docks vesicular cargo to kinesin-1.** *Mol. Biol. Cell* 2006, **17**:3651–3663.

10. Araki Y, Kawano T, Taru H, Saito Y, Wada S, Miyamoto K, Kobayashi H, Ishikawa HO, Ohsugi Y, Yamamoto T, et al.: **The novel cargo Alcadein induces vesicle association of kinesin-1 motor components and activates axonal transport.** *EMBO J.* 2007, **26**:1475–1486.

11. Pu J, Schindler C, Jia R, Jarnik M, Backlund P, Bonifacino JS: **BORC, a Multisubunit Complex that Regulates Lysosome Positioning**. *Dev Cell* 2015, **33**:176–188.

12. Rosa-Ferreira C, Munro S: **Arl8 and SKIP act together to link lysosomes to kinesin-1.** *Dev Cell* 2011, **21**:1171–1178.

13. Pu J, Keren-Kaplan T, Bonifacino JS: **A Ragulator–BORC interaction controls lysosome positioning in response to amino acid availability**. *J. Cell Biol.* 2017, **216**:4183–4197.

14. Matanis T, Akhmanova A, Wulf P, Del Nery E, Weide T, Stepanova T, Galjart N, Grosveld F, Goud B, De Zeeuw CI, et al.: **Bicaudal-D regulates COPI-independent Golgi-ER transport by recruiting the dynein-dynactin motor complex.** *Nat Cell Biol* 2002, **4**:986–992.

15. Pylypenko O, Welz T, Tittel J, Kollmar M, Chardon F, Malherbe G, Weiss S, Michel CIL, Samol-Wolf A, Grasskamp AT, et al.: **Coordinated recruitment of Spir actin nucleators and myosin V motors to Rab11 vesicle membranes.** *Elife* 2016, **5**:213.

16. Wei Z, Liu X, Yu C, Zhang M: **Structural basis of cargo recognitions for class V myosins**. *Proceedings of the National Academy of Sciences* 2013, **110**:11314–11319.

17. Wu XS, Rao K, Zhang H, Wang F, Sellers JR, Matesic LE, Copeland NG, Jenkins NA, Hammer JA III: **Identification of an organelle receptor for myosin-Va**. *Nat Cell Biol* 2002, **4**:271–278.

18. Fukuda M, Kuroda TS, Mikoshiba K: **Slac2-a/melanophilin, the missing link between Rab27 and myosin Va: implications of a tripartite protein complex for melanosome transport.** *J. Biol. Chem.* 2002, **277**:12432–12436.

19. Pylypenko O, Attanda W, Gauquelin C, Lahmani M, Coulibaly D, Baron B, Hoos S, Titus MA, England P, Houdusse AM: **Structural basis of myosin V Rab GTPase-dependent cargo recognition.** *Proc. Natl. Acad. Sci. U.S.A.* 2013, **110**:20443–20448.

20. Li X-D, Ikebe R, Ikebe M: **Activation of myosin Va function by melanophilin, a specific docking partner of myosin Va.** *J. Biol. Chem.* 2005, **280**:17815–17822.

21. Yao L-L, Cao Q-J, Zhang H-M, Zhang J, Cao Y, Li X-D: **Melanophilin Stimulates Myosin-5a Motor Function by Allosterically Inhibiting the Interaction between the Head and Tail of Myosin-5a.** *Sci. Rep.* 2015, **5**:10874.

22. Pernigo S, Lamprecht A, Steiner RA, Dodding MP: **Structural Basis for Kinesin-1:Cargo Recognition**. *Science* 2013, **340**:356–359.

23. Pernigo S, Chegkazi MS, Yip YY, Treacy C, Glorani G, Hansen K, Politis A, Bui S, Dodding MP, Steiner RA: **Structural basis for isoform-specific kinesin-1 recognition of Y-acidic cargo adaptors.** *Elife* 2018, **7**.

24. Kawano T, Araseki M, Araki Y, Kinjo M, Yamamoto T, Suzuki T: **A small peptide sequence is sufficient for initiating kinesin-1 activation through part of TPR region of KLC1.** *Traffic* 2012, **13**:834–848.

25. Dodding MP, Mitter R, Humphries AC, Way M: **A kinesin-1 binding motif in vaccinia virus that is widespread throughout the human genome.** *EMBO J.* 2011, **30**:4523–4538.

26. Farías GG, Guardia CM, Britt DJ, Guo X, Bonifacino JS: **Sorting of Dendritic and Axonal Vesicles at the Pre-axonal Exclusion Zone**. *Cell Rep* 2015, **13**:1221–1232.

27. Blasius TL, Cai D, Jih GT, Toret CP, Verhey KJ: **Two binding partners cooperate to activate the molecular motor Kinesin-1**. *J. Cell Biol.* 2007, **176**:11–17.

28. Yip YY, Pernigo S, Sanger A, Xu M, Parsons M, Steiner RA, Dodding MP: **The light chains of kinesin-1 are autoinhibited.** *Proc. Natl. Acad. Sci. U.S.A.* 2016, **113**:2418–2423.

29. Sanger A, Yip YY, Randall TS, Pernigo S, Steiner RA, Dodding MP: **SKIP controls lysosome positioning using a composite kinesin-1 heavy and light chain-binding domain.** *Journal of Cell Science* 2017, **130**:1637–1651.

30. Cockburn JJB, Hesketh SJ, Mulhair P, Thomsen M, O'Connell MJ, Way M: **Insights into Kinesin-1 Activation from the Crystal Structure of KLC2 Bound to JIP3**. *Structure* 2018, **26**:1486–1498.e6.

31. Sun T, Li Y, Li T, Ma H, Guo Y, Jiang X, Hou M, Huang S, Chen Z: **JIP1 and JIP3 cooperate to mediate TrkB anterograde axonal transport by activating kinesin-1.** *Cell. Mol. Life Sci.* 2017, **74**:4027–4044.

32. Hammond JW, Griffin K, Jih GT, Stuckey J, Verhey KJ: **Co-operative Versus Independent Transport of Different Cargoes by Kinesin-1**. *Traffic* 2008, **9**:725–741.

33. Fu M-M, Holzbaur ELF: **JIP1 regulates the directionality of APP axonal transport by coordinating kinesin and dynein motors.** *J. Cell Biol.* 2013, **202**:495–508.

34. Sun F, Zhu C, Dixit R, Cavalli V: **Sunday Driver/JIP3 binds kinesin heavy chain directly and enhances its motility.** *EMBO J.* 2011, **30**:3416–3429.

35. Raiborg C, Wenzel EM, Pedersen NM, Olsvik H, Schink KO, Schultz SW, Vietri M, Nisi V, Bucci C, Brech A, et al.: **Repeated ER–endosome contacts promote endosome translocation and neurite outgrowth**. *Nature* 2015, **520**:234–238.

36. Pankiv S, Alemu EA, Brech A, Bruun J-A, Lamark T, Øvervatn A, Bjørkøy G, Johansen T: **FYCO1 is a Rab7 effector that binds to LC3 and PI3P to mediate microtubule plus end-directed vesicle transport.** *J. Cell Biol.* 2010, **188**:253–269.

37. Urnavicius L, Zhang K, Diamant AG, Motz C, Schlager MA, Yu M, Patel NA, Robinson CV, Carter AP: **The structure of the dynactin complex and its interaction with dynein.** *Science* 2015, **347**:1441–1446.

38. McKenney RJ, Huynh W, Tanenbaum ME, Bhabha G, Vale RD: **Activation of**

cytoplasmic dynein motility by dynactin-cargo adapter complexes. *Science* 2014, **345**:337–341.

39. Schlager MA, Hoang HT, Urnavicius L, Bullock SL, Carter AP: **In vitro reconstitution of a highly processive recombinant human dynein complex**. *EMBO J.* 2014, doi:10.15252/embj.201488792.

40. Urnavicius L, Lau CK, Elshenawy MM, Morales-Rios E, Motz C, Yildiz A, Carter AP: **Cryo-EM shows how dynactin recruits two dyneins for faster movement.** *Nature* 2018, **554**:202–206.

41. Lee I-G, Olenick MA, Boczkowska M, Franzini-Armstrong C, Holzbaur ELF, Dominguez R: **A conserved interaction of the dynein light intermediate chain with dynein-dynactin effectors necessary for processivity.** *Nat Comms* 2018, **9**:986.

42. Horgan CP, Hanscom SR, Jolly RS, Futter CE, McCaffrey MW: **Rab11-FIP3 links the Rab11 GTPase and cytoplasmic dynein to mediate transport to the endosomal-recycling compartment.** *Journal of Cell Science* 2010, **123**:181–191.

43. Grotjahn DA, Chowdhury S, Xu Y, McKenney RJ, Schroer TA, Lander GC: **Cryo-electron tomography reveals that dynactin recruits a team of dyneins for processive motility.** *Nat Struct Mol Biol* 2018, **25**:203–207.

44. Grigoriev I, Splinter D, Keijzer N, Wulf PS, Demmers J, Ohtsuka T, Modesti M, Maly IV, Grosveld F, Hoogenraad CC, et al.: **Rab6 regulates transport and targeting of exocytotic carriers.** *Dev Cell* 2007, **13**:305–314.

45. Splinter D, Tanenbaum ME, Lindqvist A, Jaarsma D, Flotho A, Yu KL, Grigoriev I, Engelsma D, Haasdijk ED, Keijzer N, et al.: **Bicaudal D2, dynein, and kinesin-1 associate with nuclear pore complexes and regulate centrosome and nuclear positioning during mitotic entry.** *PLoS Biol.* 2010, **8**:e1000350.

46. Bielska E, Schuster M, Roger Y, Berepiki A, Soanes DM, Talbot NJ, Steinberg G: **Hook is an adapter that coordinates kinesin-3 and dynein cargo attachment on early endosomes.** *J. Cell Biol.* 2014, **204**:989–1007.

47. McGuire JR, Rong J, Li S-H, Li X-J: **Interaction of Huntingtin-associated protein-1 with kinesin light chain: implications in intracellular trafficking in neurons.** *J. Biol. Chem.* 2006, **281**:3552–3559.

48. Johansson M, Rocha N, Zwart W, Jordens I, Janssen L, Kuijl C, Olkkonen VM, Neefjes J: **Activation of endosomal dynein motors by stepwise assembly of Rab7-RILP-p150Glued, ORP1L, and the receptor lll spectrin**. *J. Cell Biol.* 2007, **176**:459–471.

49. Montagnac G, Sibarita J-B, Loubéry S, Daviet L, Romao M, Raposo G, Chavrier P: **ARF6 Interacts with JIP4 to Control a Motor Switch Mechanism Regulating Endosome Traffic in Cytokinesis**. *Current*

Biology 2009, **19**:184–195.

50. Lu Q, Yu J, Yan J, Wei Z, Zhang M: **Structural basis of the myosin X PH1(N)-PH2-PH1(C) tandem as a specific and acute cellular PI(3,4,5)P(3) sensor.** *Mol. Biol. Cell* 2011, **22**:4268–4278.

51. Hirano Y, Hatano T, Takahashi A, Toriyama M, Inagaki N, Hakoshima T: **Structural basis of cargo recognition by the myosin-X MyTH4-FERM domain.** *EMBO J.* 2011, **30**:2734–2747.

52. Wei Z, Yan J, Lu Q, Pan L, Zhang M: **Cargo recognition mechanism of myosin X revealed by the structure of its tail MyTH4-FERM tandem in complex with the DCC P3 domain.** *Proc. Natl. Acad. Sci. U.S.A.* 2011, **108**:3572–3577.

53. Klopfenstein DR, Vale RD: **The lipid binding pleckstrin homology domain in UNC-104 kinesin is necessary for synaptic vesicle transport in Caenorhabditis elegans.** *Mol. Biol. Cell* 2004, **15**:3729–3739.

54. Blatner NR, Wilson MI, Lei C, Hong W, Murray D, Williams RL, Cho W: **The structural basis of novel endosome anchoring activity of KIF16B kinesin.** *EMBO J.* 2007, **26**:3709–3719.

55. Klopfenstein DR, Tomishige M, Stuurman N, Vale RD: **Role of Phosphatidylinositol(4,5)bisphosphate Organization in Membrane Transport by the Unc104 Kinesin Motor**. *Cell* 2002, **109**:347–358.

56. Hoepfner S, Severin F, Cabezas A, Habermann B, Runge A, Gillooly D, Stenmark H, Zerial M: **Modulation of receptor recycling and degradation by the endosomal kinesin KIF16B.** *Cell* 2005, **121**:437–450.

57. Du W, Su QP, Chen Y, Zhu Y, Jiang D, Rong Y, Zhang S, Zhang Y, Ren H, Zhang C, et al.: **Kinesin 1 Drives Autolysosome Tubulation**. *Dev Cell* 2016, **37**:326–336.

58. Muresan V, Stankewich MC, Steffen W, Morrow JS, Holzbaur EL, Schnapp BJ: **Dynactin-dependent, dynein-driven vesicle transport in the absence of membrane proteins: a role for spectrin and acidic phospholipids.** *Molecular Cell* 2001, **7**:173–183.

59. Lorenzo DN, Badea A, Davis J, Hostettler J, He J, Zhong G, Zhuang X, Bennett V: **A PIK3C3-ankyrin-B-dynactin pathway promotes axonal growth and multiorganelle transport.** *J. Cell Biol.* 2014, **207**:735–752.

60. Randall TS, Yip YY, Wallock-Richards DJ, Pfisterer K, Sanger A, Ficek W, Steiner RA, Beavil AJ, Parsons M, Dodding MP: **A small-molecule activator of kinesin-1 drives remodeling of the microtubule network.** *Proc. Natl. Acad. Sci. U.S.A.* 2017, **114**:13738–13743.

61. Lu W, Gelfand VI: **Moonlighting Motors: Kinesin, Dynein, and Cell Polarity**. *Trends Cell Biol.* 2017, doi:10.1016/j.tcb.2017.02.005.

62. Rocha N, Kuijl C, van der Kant R, Janssen L, Houben D, Janssen H, Zwart W, Neefjes J: **Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7-RILP-p150Glued and late endosome positioning**. *J. Cell Biol.* 2009, **185**:1209–1225.

Figure Legends

Figure 1. Myosin-V and kinesin-1 can recognize short peptide motifs found in cargo adaptors using their globular cargo binding domains that can promote activity. (a) Schematic showing representation of the conformational transition of myosin-V from its cargo-free inhibited state to a form associated with Rab11 positive endosomes. Recruitment is mediated by cooperative direct interactions with Rab11 and Spir-2, which contain a globular tail binding motif (GTBM). **(b)** Overlay of crystal structures of the myosin-Va globular tail domain with GTBMs from Spir-2 (purple) and Melanophilin (green) (PDB: 4LX2 and 5JCY) **(c)** Structure based sequence alignment (from [15]) showing core GTBM from Spir-2 and MLPH highlighting hydrophobic core and basic and acidic flanks. **(d)** Schematic showing representation of the conformational transition of kinesin-1 from its cargo-free inhibited state to a form associated with lysosomes via the small GTPase Arl8 and the cargo adaptor SKIP. **(e)** Structures of the TPR domain of kinesin-1 bound to a W-acidic motif (from SKIP, KLC2, PDB: 3NFW) and a Y-acidic motif from JIP1 (KLC1, PDB: 6FUZ). **(f)** Representative W-acidic and Y-acidic sequences from several cargo adaptors.

Figure 2. Coiled-coils are used by kinesin-1, myosin V and dynein adaptors (a) Structure of the leucine zipper 2 (LZ2) region of JIP3 (green) in complex with KLC (orange) (PDB 6EJN). Relative location of the JIP1 Y-acidic (see figure 1) binding site is also highlighted (pink). **(b)** Possible modes of motor recruitment by JIP1 and JIP3. On the left, a JIP3 dimer binds a single kinesin-1 tetramer, with support of additional interactions from JIP1. On the left, JIP3 dimers allow the formation of multi-motor complex teams by cross-linking tetramers. **(c)** Structure of RILP L2 (pink) and MLPH-GTBM (green) in complex with the myosin 5A globular tail domain (orange) (PDB: 4KP3). **(d)** Schematic of dynein and dynactin in complex with an activating coiled-coil adaptor.

Figure 3. A small-molecule that targets motor-adaptor interactions in kinesin-1 alters its activity. (a) STED immunofluorescence image of HAP1 cells treated with a small molecule, Kinesore, that promotes kinesin-1 dependent remodeling of the microtubule network. Tubulin is immunostained. **(b)** Schematic showing a possible model for Kinesore induced microtubule

projectons based on promoting microtubule-microtubule sliding by kineisn-1.

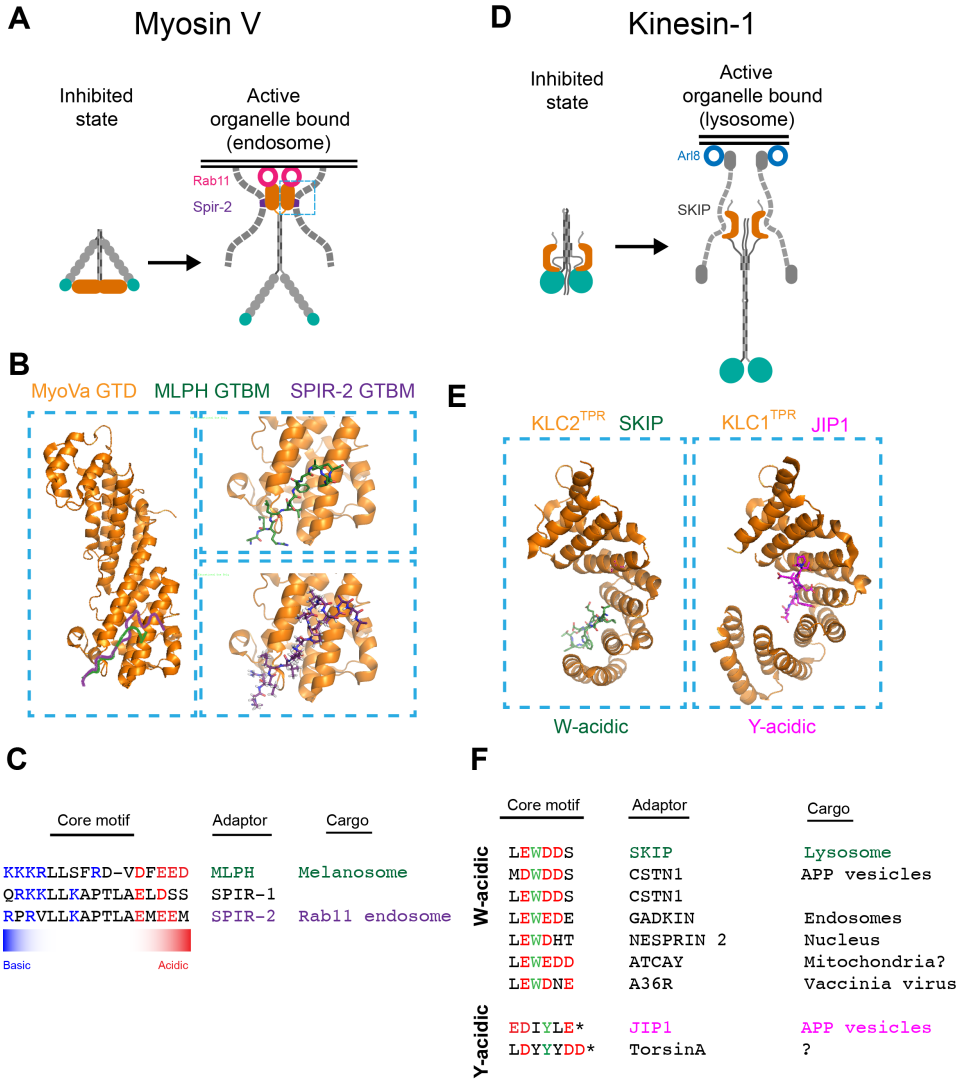

FIGURE 1.

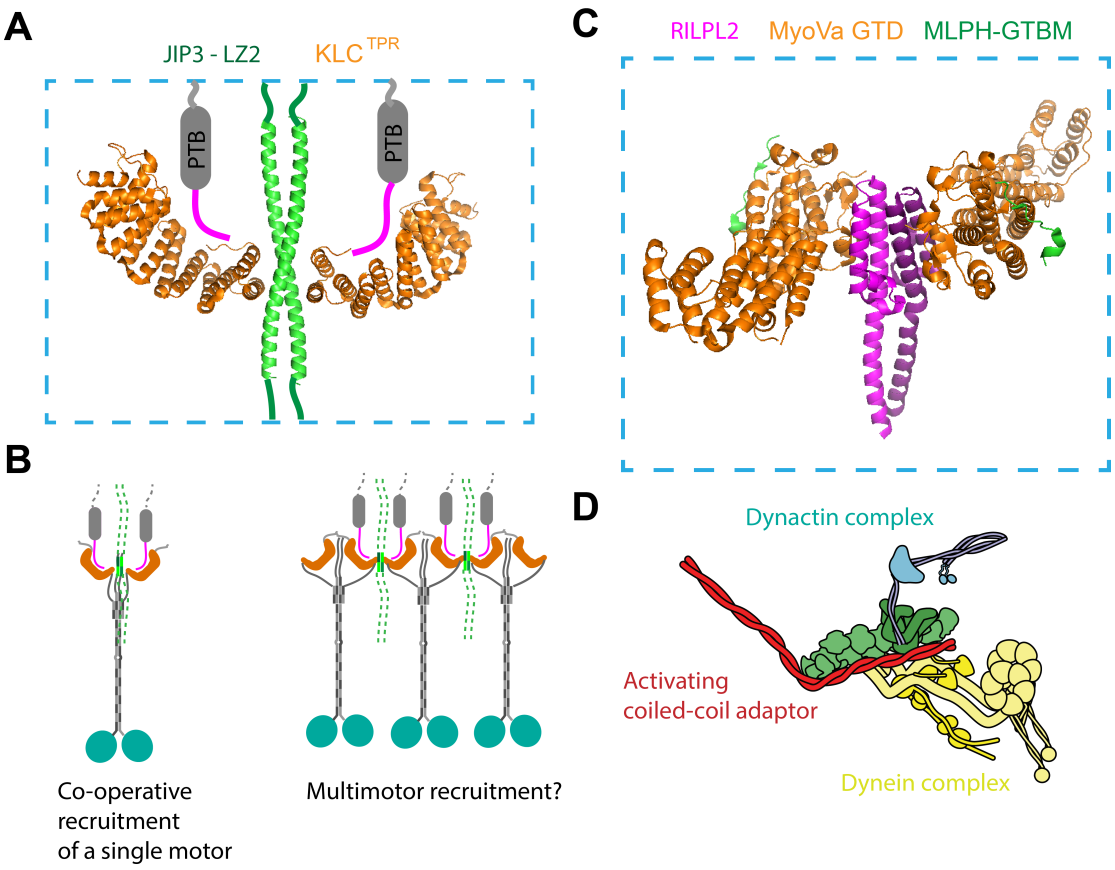
A
JIP3 - LZ2
KLC TPR
PTB
PTB
C
RILPL2
MyoVa GTD
MLPH-GTBM
B
Co-operative
recruitment
of a single motor
Multimotor recruitment?
D
Dynactin complex
Activating
coiled-coil adaptor
Dynein complex

FIGURE 2

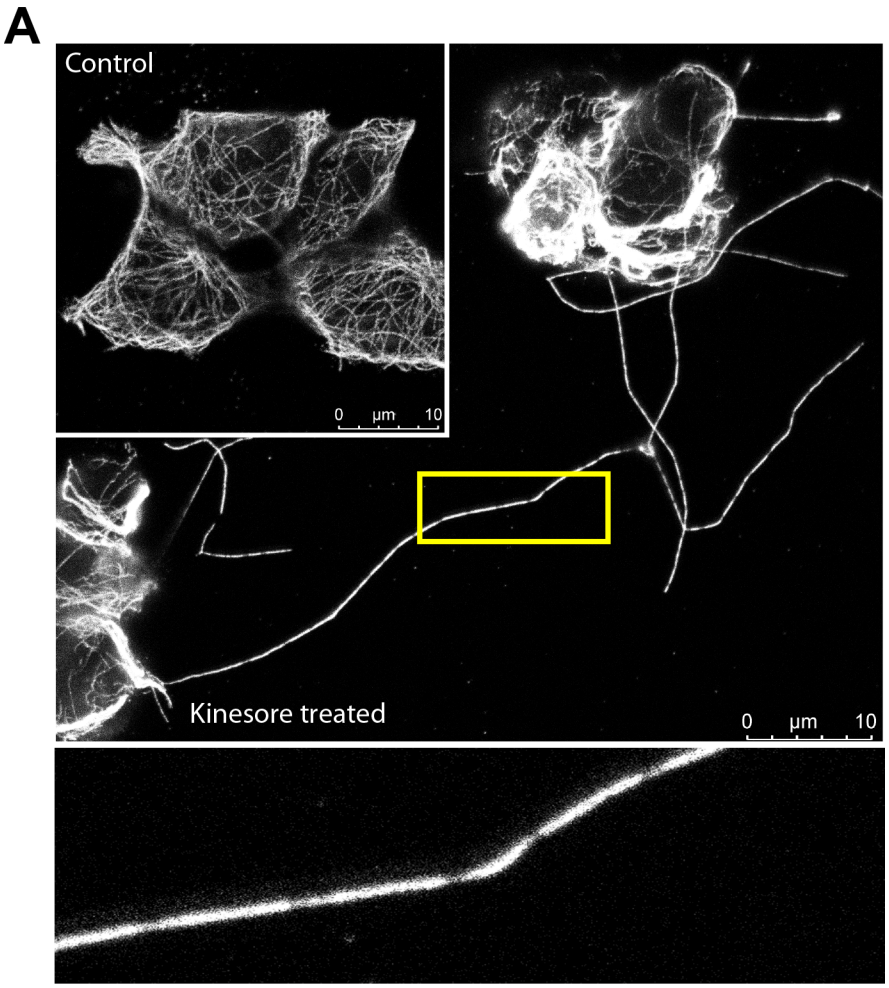

FIGURE 3